RBL-STEM Learning Activities: Analysis of Transgenic Sugarcane Development Using Artificial Neural Networks in Improving Students' Combinatorial Thinking Skills

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Abstract. Science integration, Technology, Engineering and Mathematics (STEM) in learning activities is now increasingly important. STEM approach can improve students' combinatorial thinking skills. The implementation of research-based learning together with STEM education will be a good model in designing learning activities. The background of this research is the low sugar production due to poor sugarcane productivity. Efforts to solve this problem can be done by applying biotechnology such as genetic engineering to produce high sucrose sugarcane. This study uses narrative qualitative research with a literature study method. Aim of this study is to design learning activities regarding the development of new varieties of sugarcane plants using the mutant sucrose phosphate synthase (*SoSPS1*) gene transformation technique. The results of this study describe the RBL-STEM activity framework which consists of syntax, student learning outcomes and objectives, elements of STEM, and a combinatorial skills assessment instrument framework.

Keywords: RBL · STEM · ANN · SPS · CTS

1 Introduction

Education is the primary pillar for the development and progress of the country, because it plays a role in honing abilities, knowledge, and skills. The more advanced the development of science and technology, education is required in order to improve the various skills of students in order to increase competitiveness in the future. In Indonesia, there are many human resources (HR), but in reality not many can adapt to the working world. This is because the Skills (abilities) are not matching (appropriate) as needed by the industry. For this reason, efforts are needed to improve various skills including students' combinatorial thinking skills (CTS).

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Combinatorial thinking is the thought practice of considering all alternative solutions in solving a certain problem. The combinatorial way of thinking is carried out by connecting between ideas or methods, solutions, calculations, and results [1]. Combinatorial thinking consist of five indicators [2] including: (a) identifying several cases, (b) recognizing patterns from all cases, (c) generalizing every cases, (d) systematically proving, and (e) considering with other combinatorial problems. Combinatorial thinking can build people's knowledge and experience through the process of reasoning [3]. CTS are basic skills that must be developed. Suitable strategy for developing CTS can be through the Research based learning (RBL) learning model with integration of Science, Technology, Engineering and Mathematics (STEM) approach.

The RBL learning model has been around since 2004. The development of the RBL model from its inception to the present is shown in Fig. 1. Alan Jenkins pioneered RBL learning and provided research guidance including the relationship between learning and research. Research on RBL was subsequently carried out on the effectiveness of RBL in improving meta literacy [4], critical thinking [5], problem solving [6], and metacognitive [7]. RBL is authentic learning, cooperative learning, problem solving, contextual, and based on constructivism. Research on RBL continues to be carried out as its effectiveness in improving CTS [8;9].

RBL aims to support students' knowledge development and connect research and learning, so that the knowledge gained is more meaningful. In addition, RBL can improve academic achievement, improve learning strategies, and build knowledge independently. This ability is needed, especially in the 21st century.

RBL will be more complete if it uses STEM approach which STEM skills. STEM education is known since 2003 and is an approach that emphasizes the association between real-life experiences and the concept of knowledge, so it is considered effective for achieving learning objectives. Previous research reports STEM can develop creative thinking, Meta literacy, and combinatory [10; 11; 12; 13]. The characteristics of learning that applying STEM include problem solving activities, hands-on activities, collaborative work, and multidiscipline approach. The state of art on STEM research is presented in Fig. 2.

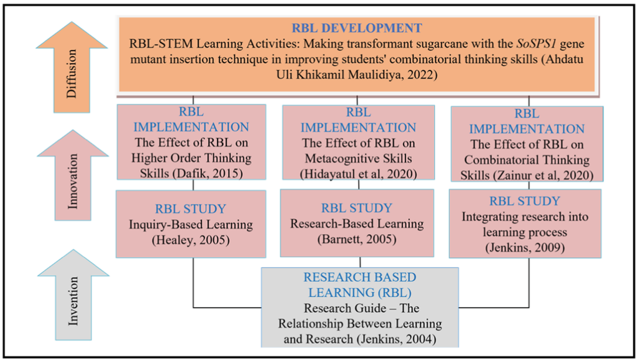

Fig. 1. State of the art on RBL model

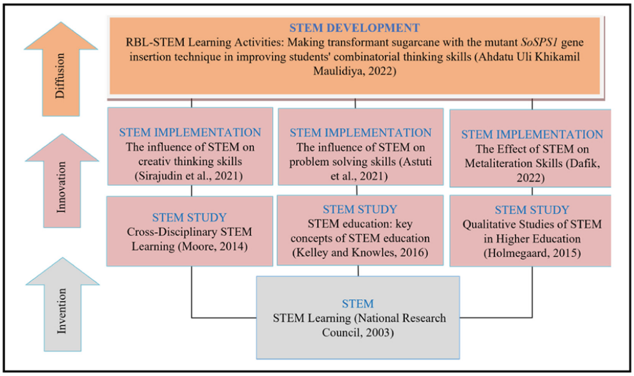

Fig. 2. State of The Art Studies STEM Learning Approaches

RBL integration with STEM aims to improve learning outcomes to improve CTS which a high-level thinking with students' ability to demonstrate multi-strategic breakthroughs and multi-solution outcomes. The development of CTS in students makes them more creative. Students not only focus to the existence of solutions, but these solutions provide varied solutions (multi-solution) to solve a problem.

The current global problem is that the low productivity of sugarcane results in less sugar production. Many efforts have been made to increase sugarcane productivity, but it does not seem to have produced encouraging results. So efforts to obtain high-yielding varieties of sugarcane are urgent to make.

The application of biotechnology seems to be the right solution in producing new cultivars for increased sugarcane growth and production. The method of genetic transformation through agrobacterium can be used to include superior traits in plants. Based on previous research, overexpression of the sucrose phosphate synthase (*SoSPS1*) gene can increase sucrose production in sugarcane [14]. Other studies report that mutants of the *SoSPS1* gene expressed in E-coli can increase the activity of SPS enzymes by 10 times [15]. Although the mutant is more stable and has prospects in the development of biotechnology, but the characterization and expression of the mutant of the N-terminal SPS domain needs to be further studied in sugarcane crops.

In the transformation process, explants such as basal plants in vitro are needed. The availability of these explants must be maintained in order to ensure the continuity of transformation. The provision of explant material through apical culture is influenced by culture conditions and culture media. Often to see the effect of the addition of plant growth regulators (PGR) on the number of in vitro shoots takes a long time. With the development of artificial intelligence and artificial neural networks, it can help predict trends in a fast time.

Therefore, in this activity we apply RBL-STEM learning in developing sugarcane crops genetically modified products sugarcane high sugar production. This study aims to design research-based learning activities with STEM approach regarding the manufacture of sugarcane transforman plants with the mutant *SoSPS1* gene insertion technique to improve students combinatorial thinking skills.

2 Research Methode

This research uses narrative qualitative research, namely developing, uncovering relevant data. The research method used is literature study, namely collecting library data, reading, and recording, and processing research materials. The data collected comes from textbooks, journals, scientific articles, and review literature containing the concepts that studied.

The steps taken include collecting literature related to RBL-STEM and then reviewing the literature. Furthermore, the development of syntactic framework for RBL-STEM integration to improve combinatorial thinking skills. Then the presentation of learning achievements and objectives along with development indicators and sub-indicators related to combinatorial thinking skills is carried out. After that sketch four STEM elements. The last step, the description of each stage of RBL equipped with learning activities, complements the indicators and sub-indicators of combinatorial skills with the help of assessment instruments.

3 Result and Discussion

3.1 Research-Based Learning Syntax (RBL) with STEM Approach

The framework for integrating the RBL-STEM learning model is designed in such a way as to increase students' combinatorial thinking skills in the problem of providing in vitro plants as transformation explanatory materials. The framework was developed based on previous research by [16]. The framework begins by posing problems arising from open problems. Open problems will spur students' skills in combinatorial thinking. This research is focused on making sugarcane transforman with the *SoSPS1* gene mutant insertion technique. A detailed STEM-integration framework can be seen in Fig. 3.

In this study, RBL-STEM model framework consisted of six steps. The first step is a fundamental question relating to the use of biotechnology in agriculture to create

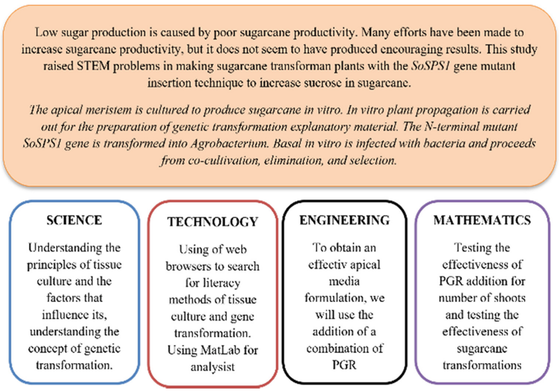

Fig. 3. STEM problems in making transformant sugarcane plants with the mutant *SoSPS1* gene insertion technique

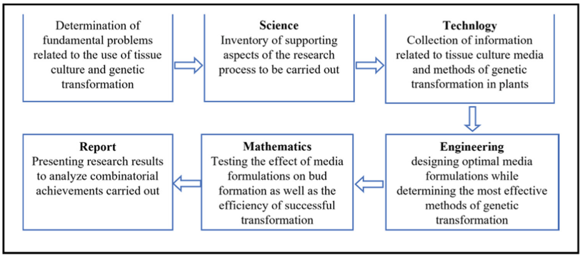

Fig. 4. RBL-STEM framework for making transgenic of sugarcane

transforming plants of the *SoSPS1* gene. The second step is an inventory of the aspects that support the research process. The third step is the collection of literacy data to understand the research process from the web or YouTube. The fourth step is to design the optimal media formulation to produce plants in invitro while determining the gene with the most effective explants in carrying out genetic transformations in plants. The fifth step is to generalize by measuring and calculating the best PGR concentration in producing in vitro plants as well as the efficiency of transformation success in producing transforming plants. The sixth step is reporting the results of research to observe students' combinatorial abilities. The RBL-STEM integration framework for making sugarcane transforming mutants of the *SoSPS1* gene is shown in Fig. 4.

3.2 Student Learning Outcomes and Objectives

The expected learning outcomes include two basic things, those are students can develop the most effective plant media formulation design with the addition of PGR's to increase the number of in vitro plant shoots of sugarcane and students can make sugarcane transformant mutant genes *SoSPS1*. RBL-STEM learning objectives here are to help students gain knowledge and abilities in the subject areas of Sciences, technology, engineering, and math. The learning objectives in detail are outlined as follows.

Science - after carrying out the research process, students are expected to be able to.

- Understand the potential of biotechnology applications that can increase sugarcane productivity.
- Understand the principles and concepts of tissue culture and techniques in the application of tissue culture.
- Understand the composition of the media needed in tissue culture and how to obtain an effective medium for bud formation
- Understanding how genetic engineering works through genetic transformation in sugarcane

Technology - after carrying out the research process, students are expected to be able to:

- Using a web browser to figure out how to create a suitable medium for tissue culture so that it is effective in bud formation.
- Using a YouTube channel to find out how to create media, planting apical cultures, and how to transform genetics in sugarcane.

- Utilizing the Matlab application to analyze the effectiveness and efficiency of bud formation using artificial neural network analysis techniques

Engineering - after carrying out the research process, students are expected to be able to:

- Design a basal media formulation with the addition of PGR and prepare explant plantings to induce apical buds.
- Carry out the process of creating media based on pre-made designs
- Perform a simulation process using Matlab and find the best combination to produce accurate data.

Mathematics - after carrying out the research process, students are expected to be able to:

- Calculating formulations in the manufacture of basal media, including the manufacture of Murhashige and Skoog (MS) media stocks, the amount of vitamins, sucrose, gelrite, and PGR concentrations that used, as well as calculating the pH in the media.
- Perform calculations on the number of buds formed
- Perform a calculation of the number of shoots using ANN in the Matlab application
- Studying the calculation of the efficiency of transformation of sugarcane.

3.3 Making Transgenic Sugarcane Plants with Mutant SoSPS1 Gene Insertion Technique

a) *Elements of science:* Sugarcane is a sugar-producing crop. The low productivity of sugarcane causes sugar production to decrease. Conventional sugarcane propagation has many shortcomings, including requiring a lot of seedlings, a large space, and its non-uniform nature. For this reason, options are needed that will be used as a breakthrough in solving these problems, for example in the field of biotechnology. The growing curiosity will encourage students to look for references via the web or YouTube. Here students will discover and build their own knowledge that biotechnology applications can improve plant properties through tissue culture and transformation Fig. 5. The success of tissue culture is influenced by various factors such as plant nutrition. Adequate nutrition can increase the success of tissue culture. Similarly, in genetic transformation, different methods can affect the success of transformation.

b) *Elements of technology:* Along with the development of technology, the internet plays an important role in the world of education. The internet acts as a source of information and data so that students can use it for learning activities. Web browsers, YouTube, journals, and various other applications can be used as sources of insight and knowledge. Through the web and YouTube, students can use it as a source of literacy for making transgenic sugarcane plants Fig. 6.

On the other hand, the use of the Matlab application can help predict the number of shoot formation based on input data that generated from the addition of PGR's (BAP, Kinetin, and GA) (Fig. 7).

c) *Element of engineering:* To make the Mutant sugarcane SoSPS1 gene requires two techniques. The first is the technique of providing in vitro plants that are used as explant preparations for transformation which include media manufacturing techniques

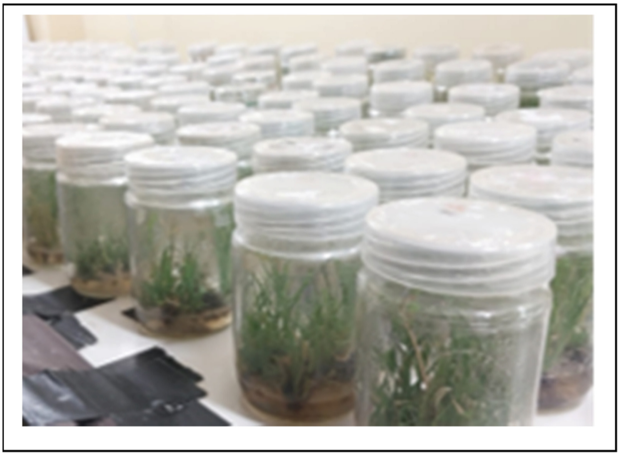

Fig. 5. Sugarcane apical culture

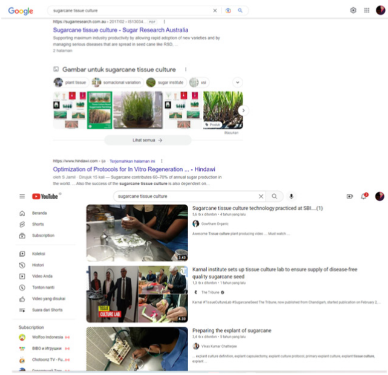

Fig. 6. Web browser and youtube for making transgenic sugarcane plants

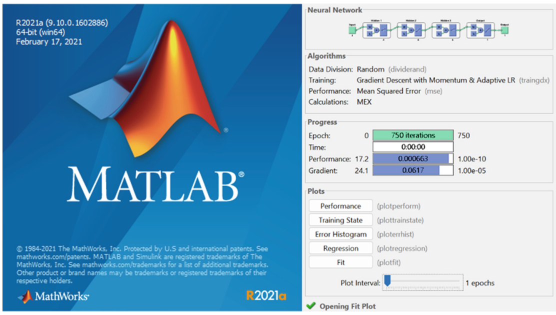

Fig. 7. Using of Matlab application for analysis of in vitro shoot number on sugarcane

with the addition of different concentrations of PGR, sterilization techniques for tools, spaces, and explants, initiation techniques, and regeneration techniques. The tools and materials used in this technique include: murhasige and skoog (MS) media, PGR's (BA, Kinetin, Giberilin), and a set of tissue culture tools. The second is the technique of transforming the SoSPS1 gene mutant in sugarcane which includes explant preparation techniques, transformation techniques to bacteria and plasmid isolation, infection techniques, and transforman plant screening techniques Fig. 8. Tools and materials for plant transformation include: in vitro of sugarcane, media (YEP, co-cultivation, elimination, and selection), Antibiotics (kanamycin, gentamicin, cefotaxime), Plasmid purification kits, Polymeration chain reaction (PCR), and Geldoc system.

d) *Elements of mathematics:* In the mathematical element, the number of buds formed based on the addition of different PGR (BAP, Kinetin, and GA) to the media will be studied. An overview of the third combination of PGR is presented in Table 1 and variations are obtained from the yield of shoot formation. So a technique is needed to predict data targets, for example by using artificial neural networks. The optimal on the substrate is carried out by counting the number of formed buds. The results of the calculation of the number of shoot from various PGR (BAP, Kinetin, and GA) are presented in Table 1.

ANN is an artificial neural network in the form of a processing unit that modeled based on the human nervous system. The ANN architecture is made like a brain structure composed of neurons that are interconnected with each other. A dendrite, a cell body, and an axon are the components of a human neuron. Dendrites are signal receivers and are affected by weight. The cell body where the incoming and weight signals are computed produces an output signal that will be transmitted to other neurons. Whereas axon sends an output signal to other neurons connected to it. From the description, ANN can be presented into three parts, namely the input, the output, and the hidden layer that transforms data coming from the input layer into something the output layer can accept.

Fig. 8. Methods of plant transformation

Table 1. Calculation of the number of shoots on sugarcane

BAP	*KIN*	*GA*	*Number of shoots*
1	0,1	0,1	26
1,5	0,1	0,1	24
2	0,1	0,1	18
3	0,1	0,1	17
0,25	0,25	0,1	30
0,5	0,25	0,1	29
1	0,25	0,1	23
1	0,5	0,1	24
1	0,1	0,25	28
1	0,1	0,5	30

Table 2. Comparison of effectiveness between multiple models and network architectures

ANN Model	**ANN Architecture**	**Regression Train**	**Time train**	**MSE Training**	**MSE Test**
Feed forward net	ANN-(a)567 ANN-(a)745 ANN-(a)466	0.99998 0.99973 0.99993	0.7820 0.7180 0.6880	0.0008724 0.01045 0.0025995	0.0009 0.0105 0.0026
Fitnet	ANN-(a)567 ANN-(a)745 ANN-(a)466	0.99997 0.99998 0.99998	0.8733 0.7140 0.7090	0.0013337 0.0007770 0.0006634	0.0013 0.0008 0.0007
Cascade forwardnet	ANN-(a)567 ANN-(a)745 ANN-(a)466	0.99933 0.99982 0.99998	1.0470 0.8270 0.8420	0.026426 0.0070173 0.0008679	0.0264 0.0070 0.0009

How ANN works is like a human being, if humans learn based on experience, then machines learn based on data. As much as 70% of the input data and target data in training so that the machine learns to produce accurate weight vectors. Furthermore, to see the accuracy of testing, 30% of the testing data was carried out. If accurate, it can be used to predict any new input data in this case, namely related to the number of buds formed.

Matlab programing is carried out with three input data in the form of adding PGR's (BAP, Kinetin, and GA) to the output data, namely the number of shoots formed. Here we used three ANN Architecture (466, 567, 745) with three ANN models (Feedforwardnet, Fitnet, Cascadeforwardnet) and analyzed further to get the most optimum model. The best parameter results of all three models and architectures are shown in Table 2.

In order to choose the most optimal model and architecture is selected based on the most minimum MSE. Fitnet's model (4.6.6) gives a regression of 0.99998, with both

Table 3. RBL- STEM MODEL LEARNING ACTIVITY STAGE 1

Stage 1	**Activity**
Determination of fundamental problems related to the use of tissue culture and genetic transformation	1. Lecturer presents data on the need for sugar in Indonesia that is greater than its production. 2. Lecturers will ask students if they know about the application of biotechnology in agriculture? What are the applications of biotechnology that can be used to improve plant properties? 3. Lecturers will ask students if they know about tissue culture? What is it principle? What are the techniques in tissue culture? 4. Lecturers will present photos and videos as background and introduce about the stages in tissue culture and gene transformation in sugarcane plants.

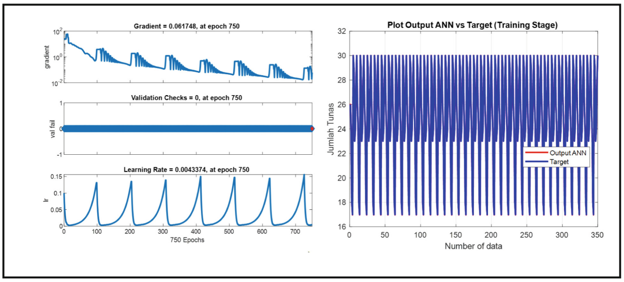

Fig. 9. Programming results related to learning rate and testing training data between target and output

MSE training and MSE testing of 0.0006634 being the most optimal models. This value indicates the compatibility between the network output and the target. If the regression value approaches one (1), then the network output is more appropriate. So that the results of the regression values that have been obtained show that the network has been able to approach the target value.

From these results, there is an interpolation of output data almost resembling target data throughout the testing data on a comparison graph between target data and output data Fig. 9. This means that the machine learns well and can be used to predict any similar data related to the number of buds formed. Machine learning simulations in forecasting 30% of input data without being given target data are seen in Fig. 10 and the results can draw riel conditions that can be shown in graphs with abscissa ranging from 150 to 200. The regression coefficient values and MSE values that generated during the testing process showed that an ANN with an architecture of 466 was good enough to predict the number of buds formed. Small MSE value indicates that the error factor is also small.

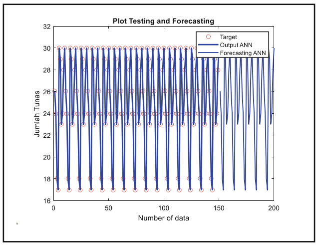

Fig. 10. Programming results related to testing data and comparison between target and output

The ANN method can be used to forecast the number of buds, thus assisting in making decisions regarding the selection of PGR and the most appropriate concentration.

3.4 RBL-STEM Learning Framework for Making Transgenic Sugarcane

The RBL-STEM framework is divided into six stages that will be adjusted one by one. This step illustrates how students carry out the learning process with the RBL-STEM model on the material for making sugarcane transformant mutant *SoSPS1* genes in an effort to improve students' combinatorial abilities.

a) Phase 1 submission of fundamental issues with sugarcane transformant mutant SoSPS1 gene. The learning activities that will be carried out by lecturers and students are described in more detail in Table 1.

b) Second stage (science) is in the form of developing breakthroughs related to making Transforman sugarcane by injecting the SoSPS1 gene in sugarcane to increase students' combinatorial thinking skills. The lecturer asked the class to list some of the supplies and equipment required to create transgenic sugarcane. For more details, it is described in Table 4.

c) This third stage is utilizing of technology or software in data search in the form of using the internet of things to understand more deeply about the manufacture of transgenic sugarcane mutant SoSPS1 gene: determination of explant materials, media formulations for explant propagation, transformation techniques, and transformant plant screening. Learning activities at this stage are outlined in Table 5.

d) The engineering stage contains the core process of research on RBL-STEM learning. This stage is carried out by designing an effective media formulation for the formation of in vitro buds while determining the most effective method of carrying out genetic transformations in plants. Those two activities in the implementation of students are divided into two groups to do. Description of the engineering is presented in Table 6.

e) The fifth stage is to generalize the research results by measuring and calculating the best PGR concentration in producing in vitro plants and the efficiency of successful transformation in producing transformant plants. Calculations can be done manually, excel, and Analysis of Variance (ANOVA) on SPSS followed by BNT test. In addition, for data simulation, you can use the Matlab application which refers to the neural network, so that it can predict output quickly based on the inputted data as Table 7. Another calculation is the transformation efficiency in producing sugarcane plants transformant mutant SoSPS1 gene.

Table 4. RBL-STEM Model Learning Activity Phase 2.

Stage 2	**Activity**
Inventorying the supporting aspects of the research process to be carried out	1. The lecturer conveyed aspects that must be understood so that the research process can run smoothly. 2. Students and their groups write down what aspects must be understood first. 3. Students and their groups share assignments to find and understand the aspects needed. 4. Students and their groups discuss what tools and materials are needed before conducting research. 5. The lecturer instructed the class to check out the procedures for creating in vitro plants and creating transformant sugarcane plants in the literature. 6. Students and their groups have an initial discussion about aspects that have been understood before completing them using internet sources

Table 5. RBL-STEM Model Learning Activity Stage 3

Stage 3	**Activity**
Collecting data and looking for sources related to tissue culture and gene transformation to understand the research process via the web or YouTube	1. Students with the guidance of lecturers search literature on tissue culture and gene transformation in sugarcane according to their respective duties. 2. Students collecting data, photos, or videos of methods in tissue culture and gene transformation in sugarcane. 3. Students are looking for literature related to media for the formation of in vitro shoots of sugarcane that will be used as explants. 4. The lecturer reviews each student's search results and offers advice if there are any remaining gaps in the information found.

f) The sixth stage is reporting the results of the study and communicating conclusions based on findings related to tissue culture and the manufacture of sugarcane plants transformant mutant SoSPS1 gene in student work sheet. First, students conducted an internal focus group discussion (FGD) which aimed to ensure that each group member was ready to explain according to their respective parts. Here researchers can observe

Table 6. RBL-STEM Model Learning Activity Stage 4

Stage 4	**Activity**
Designing optimal media formulations while determining the most effective methods of genetic transformation	1. The lecturer provides confirmation of the basic way of making tissue culture media formulations. 2. Students design media formulations with the addition of different PGR. 3. Students determine the optimal formulation for the formation of plant buds in invitro sugarcane. 4. Teacher gives confirmation of how to carry out genetic transformations 5. Students conduct research according to student work sheet. 6. Students write down the conclusions of the study.

Table 7. RBL-STEM Model Learning Activity Stage 5

Stage 5	**Activity**
Testing the effect of media formulations on bud formation as well as the efficiency of successful transformation	1. The student group started the presentation with a simulation of the measurement and calculation of the PGR concentration used. 2. Students show the results of measurements and calculations of the use of PGR in the formation of in vitro plant buds 3. Students simulate calculations using the MatLab application 4. Students show the results of the calculation of the efficiency of the transformation that has been carried out 5. Students show the results of the calculation of transformation efficiency

the combinatorial abilities of students. The description of the activities at the sixth stage is shown in Table 8.

3.5 Student Combinatorial Skills Assessment Instrument Framework

The instrument is structured to confirm learning outcomes, especially describing the process and results of students' combinatorial thinking skills. The results of the assessment instrument describe the sequential level of student combinatorial ability. The student combinatorial skills instrument assessment framework is presented in Table 9 which contains indicators, sub-indicators, and test materials.

Table 8. RBL-STEM Model Learning Activity Stage 6

Stage 6	Activity
Presenting the results of student research to analyze the combinatorial achievements carried out	1. Students carry out group internal FGDs to prepare for presentations 2. Group representative students carry out presentations by conveying the objectives and from learning activities and conclusions obtained 3. Students open Q&A session with other group members 4. Each student research project's outcomes are evaluated by lecturers. 5. Lecturers observe students' combinatorial skills using student activity observation sheets. 6. Lecturers and students together conclude the research that has been carried out.

3.6 Follow-Up Development of Learning Tools

After making the design of RBL-STEM activities, the follow-up that will be carried out is to carry out research on the creation of learning tools with the ADDIE model (analysis, design, development, implementation, and evaluation). The learning process, materials, and student characteristics were analyzed during the analysis phase. Research development will be carried out by paying attention to the university level. The design stage is carried out by designing learning tools that integrate the RBL model into a STEM approach consisting of a syllabus, RPS, MFI, pretest-posttest, and other assessment instruments. At the development stage, tests are conducted on learning tools and instruments to determine their validity and practicality. he implementation phase is used to evaluate the effectiveness of RBL-STEM learning tools to improve students' combinatorial skills.The final stage is an evaluation used to establish whether the application of RBL STEM learning tools in solving the problem of making genetic transformation for sugarcane can improve students' combinatorial abilities.

4 Discussion

The development of an RBL-STEM learning activity framework in making transformant sugarcane plants with the mutant *SoSPS1* gene insertion technique to improve combinatorial thinking skills is very important to do. These findings serve as guidance for further research. At least two further studies could be conducted, namely: (1) making RBL-STEM learning materials using the ADDIE development model and (2) analyze the implementation of RBL-STEM learning tools in improving students' combinatorial thinking skills in development of transformant in sugarcane with the mutant *SoSPS1* gene insertion technique.

In this study, Artificial Neural Networks (ANN) were used as technological elements in RBL-STEM learning. Based on the results, the ANN FitNet architecture 466 model

Table 9. Indicators Affecting Combinatorial Thinking Ability in STEM activities

Indicators	**Sub Indicators**	**Material**
Identifying multiple cases	a. Identifying the characteristics of the problem of low sugarcane productivity b. Explaining the impact of low sugarcane productivity	a. Explain why sugarcane productivity is still lacking b. Explain how low sugarcane productivity impacts sugar production
Generalizing all cases	a. Schetizing the problem solving patterns found b. Counting the number of emerging patterns c. Developing pattern syntax expansion	a. Determining the scheme of addition of PGR in tissue culture media and the scheme of methods used in genetic transformation b. Understand how to calculate the percentage of bud formation and the efficiency of transforman plants c. Developing syntax of administering different PGR on the medium and develop appropriate methods of genetic transformation
Systematically proving	a. Analyzing the advantages and disadvantages of pattern completion b. Testing the correctness of pattern expansion in general c. Developing a more general pattern mapping d. Testing the correctness of mapping more general patterns e. Applying inductive, deductive, or qualitative substantiation	a. Analysis of the advantages and disadvantages of adding PGR to the medium against the formation of in vitro buds and the disadvantages and advantages of the transformation method b. Apply predetermined media formulations with many options on tissue culture and apply various transformation methods to plants c. Measure and calculate the percentage of bud formation in each of the media that used and measure the efficiency of the various plant transformation methods that used d. Analysis the results of the effect of PGR addition on bud formation and the influence of transformation methods on transformation efficiency e. Apply more applicable findings
Considering with other combinatorial issues	a. Interpreting b. Filing open issue c. Identifying new combinatorial problems d. Finding potential applications of found settlement patterns	a. Summing up the results of the study b. Determine open issues related to screening of sugarcane plants transformant mutant *SoSPS1* gene c. Identify new issues in improving sugarcane productivity d. Discover potential applications of settlement patterns that found

provides the best value for predicting shoot formation. The use of this ANN can help quickly predict the addition of optimal PGR combinations for the formation of shoots in sugarcane. This activity framework will be effective in improving student skills if applied in learning, this is in line with the results of previous studies [2; 9]. We believe that integrating RBL-STEM for other sciences will familiarize students with finding breakthroughs towards complex problems and that is the key to creating a youthful generation that is prepared for the future, that has creative-innovative, critical, collaboration, and communication thinking skills.

5 Conclusions

We have developed an RBL-STEM learning syntax in making transformant sugarcane plants with the *SoSPS1* gene mutant insertion technique to promote students' combinatorial thinking skills. This result is a beginning activity so as to facilitate further research related to the development of tools and their implementation to students.

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